Non-neuronal modulation of epileptic activities: glial cells and inflammatory processes

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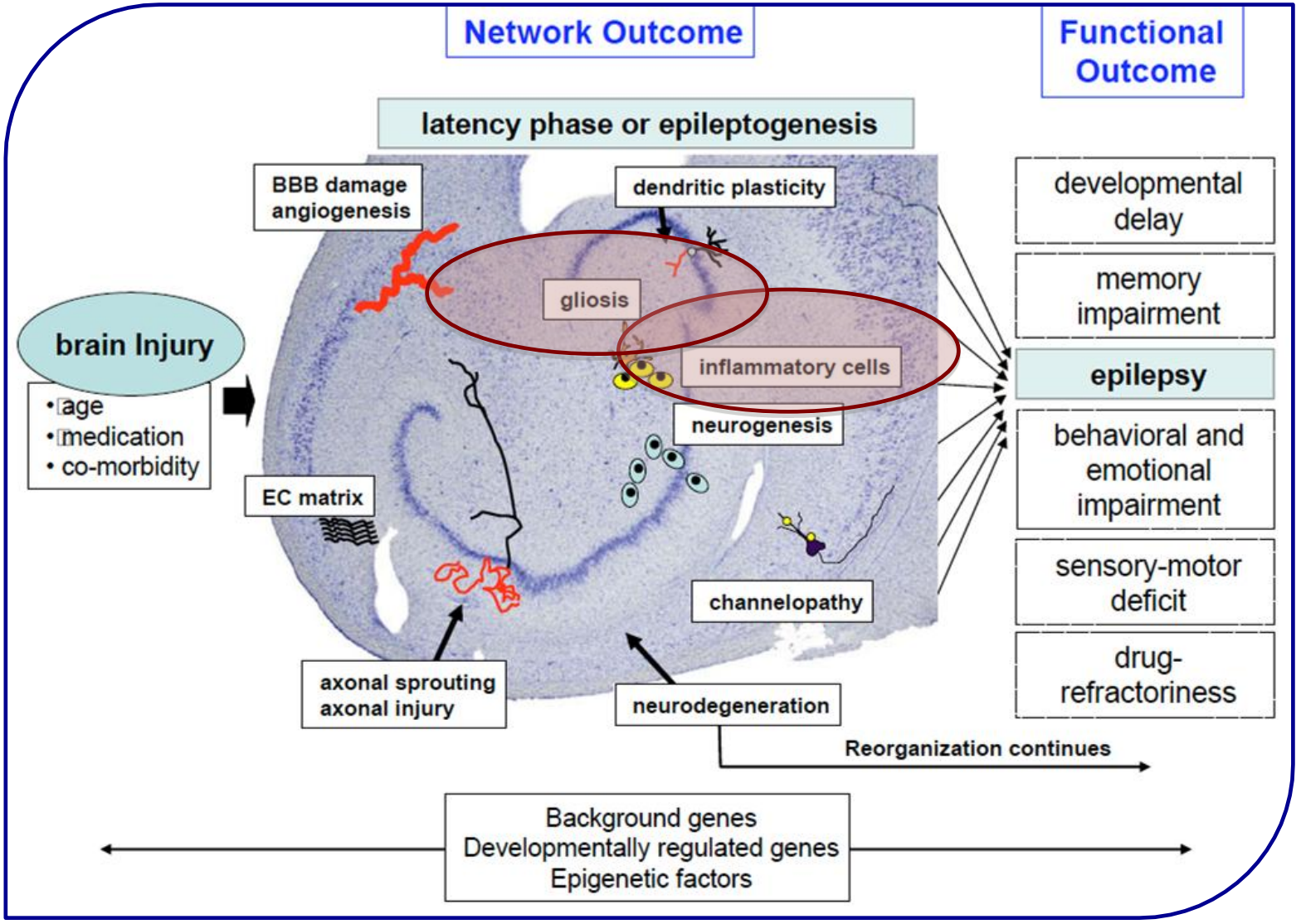
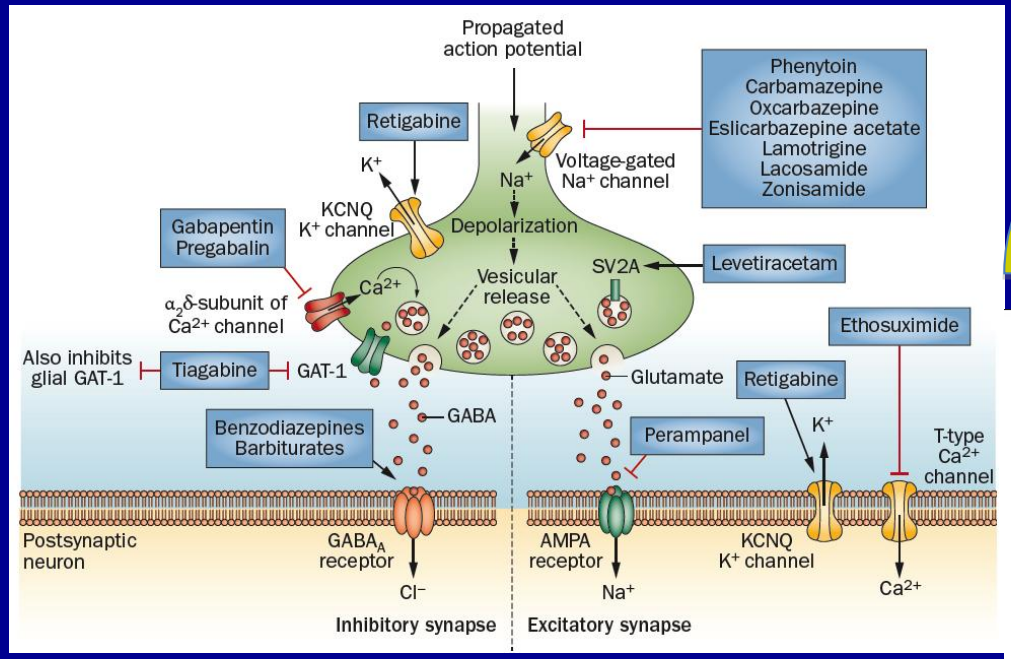
Challenge for epilepsy treatment: searching new targets for drug development

✓Treatment of resistant seizures

✓Disease-modifying drugs

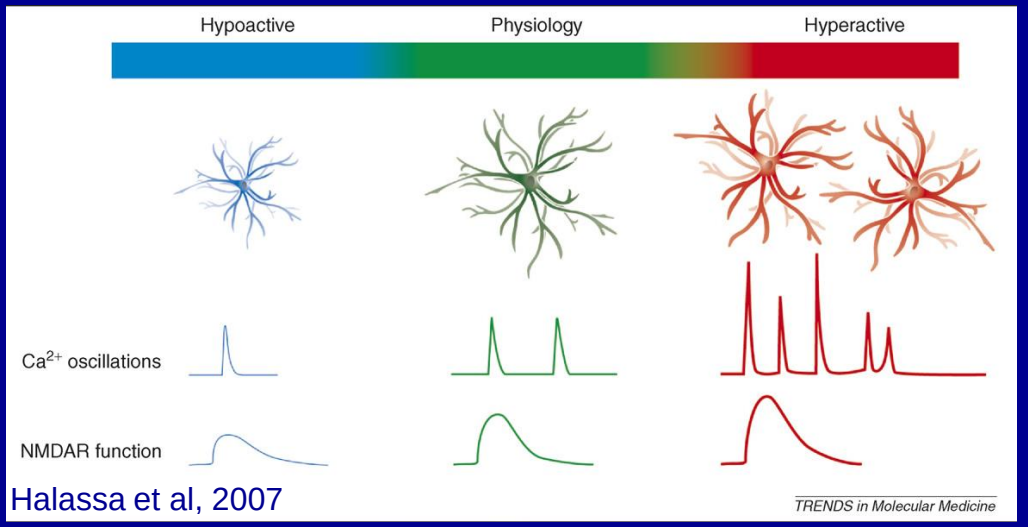


Pitkanen and Lukasiuk, Epilepsy & Behavior, 2008



Loscher & Schmidt, Nat Rev Neurol, 2012

The astrocytic activation spectrum

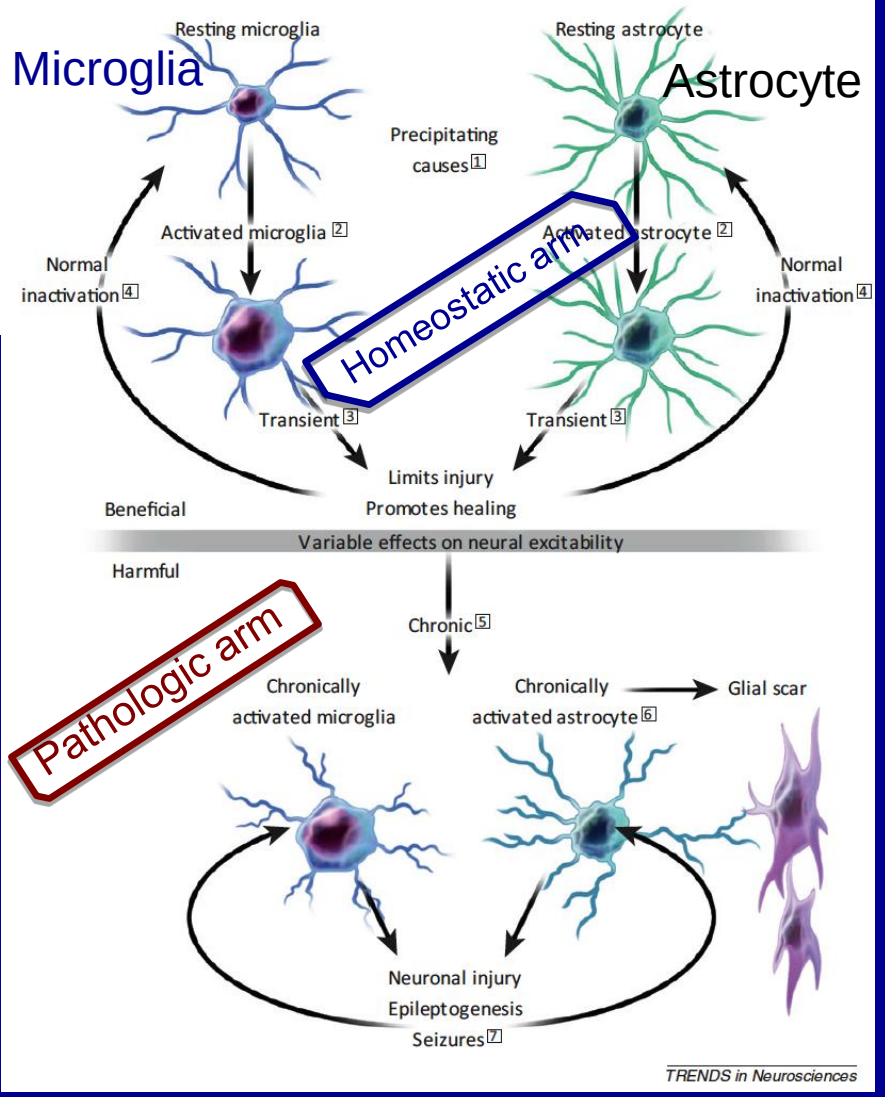


Halassa et al, 2007

Gliotransmission in health and disease

Volterra & Meldolesi, Nature Rev Neurosci, 2005;
Seifert & Steinhauser, Nature Rev Neurosci, 2006;
Perea et al, TINS, 2009; Devinsky, Vezzani et al, TINS, 2013

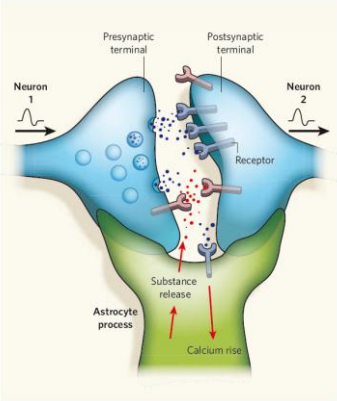
From neurons to glia



Astrocytes-Neurons interactions & epileptic activities

Astrocytes have smart communicative functions

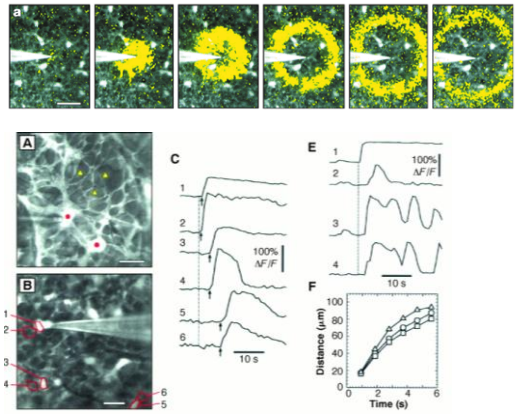
"Tripartite synapse" *(Allen Nature 2009)*



➜ *target to modulate synaptic efficacy?*

Information transmission: Ca^{++} waves

(Newman Science 1997 - Haydon 2001)



➜ *target to modulate signals processing?*

Table 1. Mechanisms of glia-mediated neuronal hyperexcitability

Inflammatory mediated			
Glia-derived proinflammatory molecules	↑ Rel		brain slice, dent, murine
IL-1R/TLR signaling in glia and in neurons	↑		TSC; *in vivo* nsgenic dels
Astrocyte glutamate transporters	↓		ocytic cell
Microglia-derived proinflammatory molecules	↑		TSC; *in vivo* al cell
BBB dysfunction	↑		t slice, ent
Multidrug transport proteins in endothelial cells and in perivascular astrocytes	↑		TSC; *in vivo* sgenic dels

Seizures, cell loss, comorbidities
A.Vezzani (IRFMN, Milano, Italy)
G.Carmignoto (Univ of Padova, Italy)
M.De Curtis (Carlo Besta Ist., Milano, Italy)
S.Auvin, Auvin Hôpital R.Debré, Paris, France)

Glia dysfunction & Gliotransmission
C.Steinhauser (Univ. Bonn, Germany)
A.Volterra (Univ Losanne, Switzerland)
A.Araque (Univ Madrid, Spain)
G. Huberfeld & N. Rouach (Paris, France)

BBB & Pharmacoresistance
A.Friedman (Ben-Gurion University Beer-Sheva, Israel)
J.Gorter (Amsterdam Univ, Amsterdam, The Netherlands)
U.Heinemann (Charité, Berlin, Germany)
H.Potschka (Univ Munich, Germany)
W.Loscher (Univ Hannover, Germany)

Human tissue
E.Aronica (AMC, Amsterdam, The Netherlands)
M.Lerner-Natoli (CNRS, Montpellier, France)
A.Becker (Univ Bonn,Germany)
C. Bien (Epilepsy Center Bethel, Bielefeld, Germany)
Novak&Hamer (University of Marburg,Germany)
A. Vincent (John Radcliffe Hospital, Oxford, UK)
J. Peltola (Univ Helsinski, Finland)
G. Huberfeld & N. Rouach (Paris, France)

Inflammation, ictogenesis & epileptogenesis

NSAID*: *celecoxib, parecoxib, aspirin*

Immunosuppressants: *fingolimod*

Anti-integrins antibodies*

Glia activation inhibitors:

minocycline

resveratrol

fingolimod

Inciting event

IL-1/TLR signaling

IL-1β, HMGB1, TNF-α, IL-6, COX-2 & complement

LPS/TLR4

Poly I:C/TLR3

Disease or Syndrome Modification

Antiepileptogenesis

Reversal of pathology

+attenuation of neuropathology

Co-morbidity modification

Prevention

Seizure modification

Cure

Frequency

Seizure duration

Seizure type

Progression

Learning and memory

Mood and behavior

Other

**Controversial results on seizures outcome*

Adapted from from A. Pitkanen, Epilepsia

Mazarati et al., 2010; Galic et al, 2008; Riazi et al, 2010

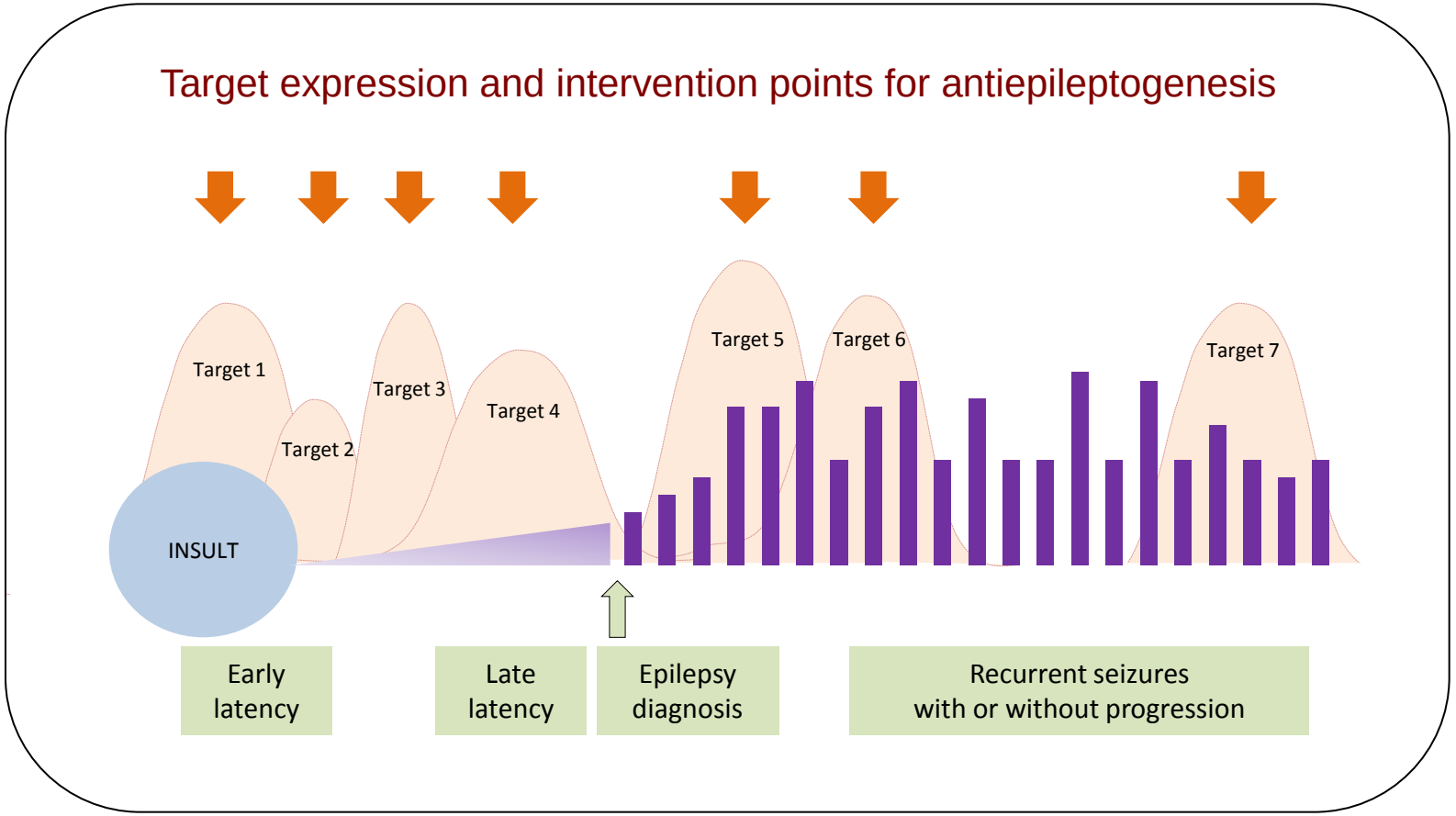
Open questions →

Finding master regulators ?
Combine treatments?
Prevention? Resolution?

(Vezzani et al, 2011; Aronica et al, 2012)

Open questions for optimizing pharmacological interventions:

- expression of inflammation-linked targets
- changes in glia activation /phenotype/priming/physiology
- experimental models: differences vs commonalities
- target validation in human specimens



Pitkanen et al, Epilepsia, 2013

Conclusions

(rele *ties)*

They *psy*

1. The

2. The
   by

3. Thei

4. Strategic therapeutic interventions to modify their function to boost beneficial clinical outcomes

Searching biomarkers of :

•glia activation
•brain inflammation
•BBB opening

(imaging, soluble mediators in CSF/blood)

Butler et al, J Neuroimaging 2010
Hirvonen et al, J Nucleic Med, 2012
Duffy et al, Neuroimage, 2012
Ravizza et al, Epilepsia, 2012

Anti-ictogenic & anticonvulsive effects

IL-1/TLR signaling

1. Seizures induced by kainic acid (lesional) or bicuculline and FS (non lesional)

(Vezzani et al, 1999; 2000; Dube' et al, 2005; 2011; Ravizza et al, 2006)

2. Status epilepticus in rats is reduced by anakinra *(De Simoni et al, 2000; Marchi et al, 2009)*

3. Electrical kindling: delayed + no seizure generalization

(Ravizza et al, 2008; Auvin et al, 2010; 2011)

4. Chronic seizures in mice (mTLE model) *(Maroso et al, 2009; 2010)*

5. SWD in GAERS & WAG/Rij rats (absence seizures) *(Akin et al, 2011; Kovács et al, 2011)*

↓

50-70% decrease in seizure recurrence, delayed seizure onset, reduced generalization

Resolution of inflammation in areas involved in seizure activity

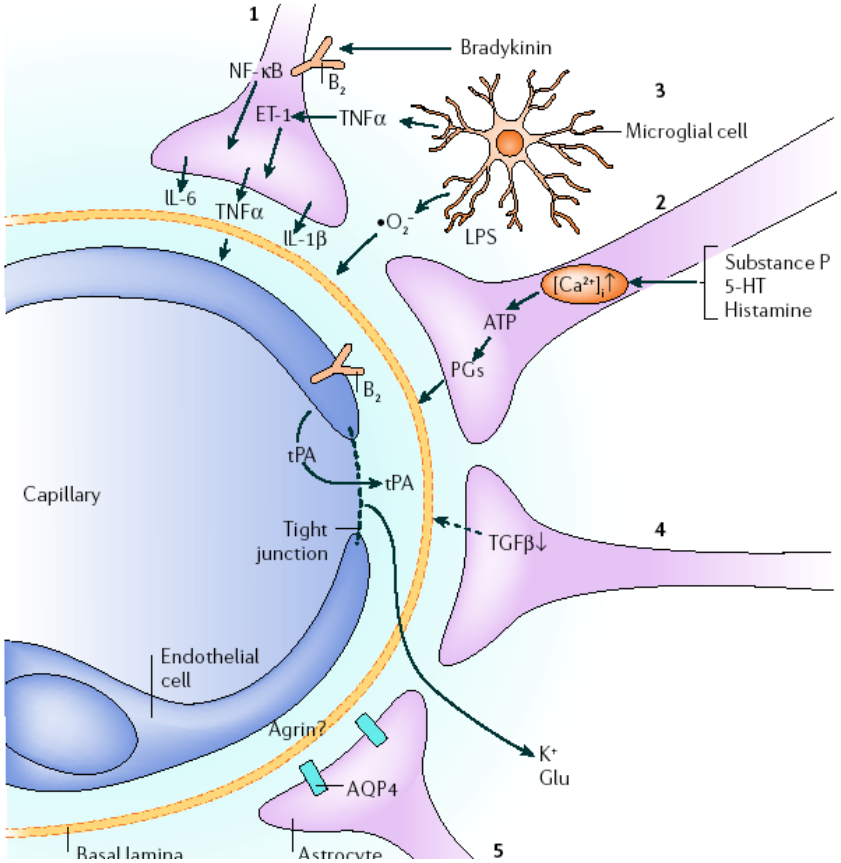
Harness these targets for pharmacological intervention

TNF-α, IL-6, COX-2 & complement system

(reviewed in Kukarni & Dhir, 2009; Vezzani et al, 2011; Aronica et al, 2012)

Perivascular glia, inflammatory mediators & brain microvasculature: New targets for intervention?

- ✓Neovascularization in CNS
- ✓Increase in BBB permeability
- ✓Induce adhesion molecules
- ✓Induce MTP involved in pharmacoresistance (e.g. P-gp)

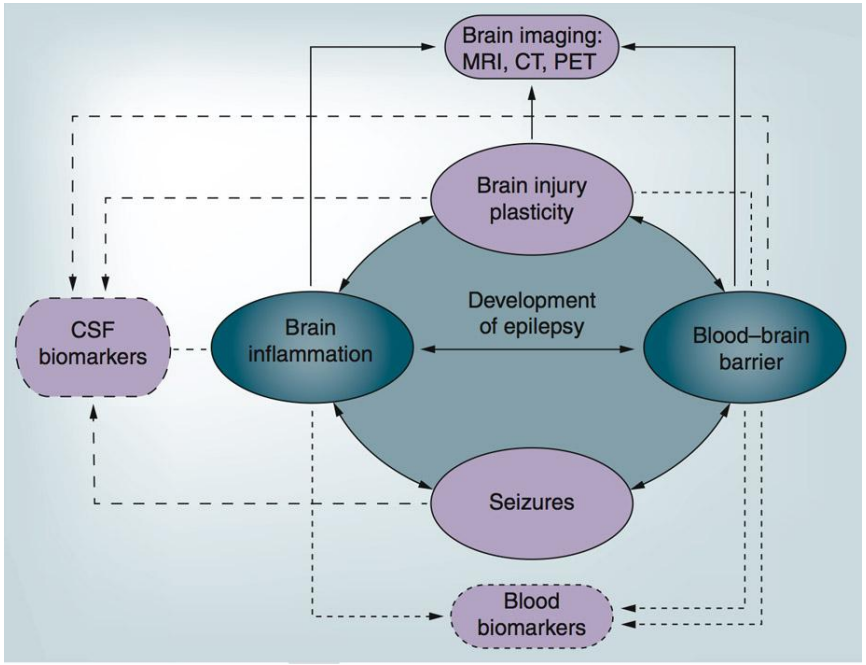


Unmet needs

Searching biomarkers of :

- •glia activation
- •brain inflammation
- •BBB opening

Butler et al, J Neuroimaging, 2010
Hirvonen et al, J Nucleic Med, 2012
Duffy et al, Neuroimage, 2012
Ravizza et al, Epilepsia, 2012



Box 1. Potential biomarkers of brain inflammation in epilepsy.

- Brain imaging (cell types or macromolecules)
  - PET (microglia/macrophages, endothelial cell adhesion molecules)
  - Magnetic resonance spectroscopy (astrocytes)
  - Molecular MRI (endothelial dysfunction; VCAM)
  - Contrast-enhanced MRI (endothelial dysfunction; increased permeability)
- Soluble inflammatory mediators in cerebrospinal fluid/blood
  - Citokines/chemokines/danger signals†
  - Cell adhesion molecules
  - Auto-antibodies
- Leukocytes
  - Cell sorting profile
  - *In vitro* responsiveness to proinflammatory challenges
  - Pro- or anti-inflammatory gene polymorphisms‡

See main text for details.
†Danger signals are endogenous molecules released from cells exposed to stressful events. For example, high-mobility group box 1 is a danger signal released from glia and neurons in epileptic tissue [34]; increased high mobility group box 1 blood levels have been measured in neurological disorders [72].
‡A modest association between the IL-1β gene and epileptic disorders has been reported [73,74].

Vezzani and Friedman, Biomark Med, 2011

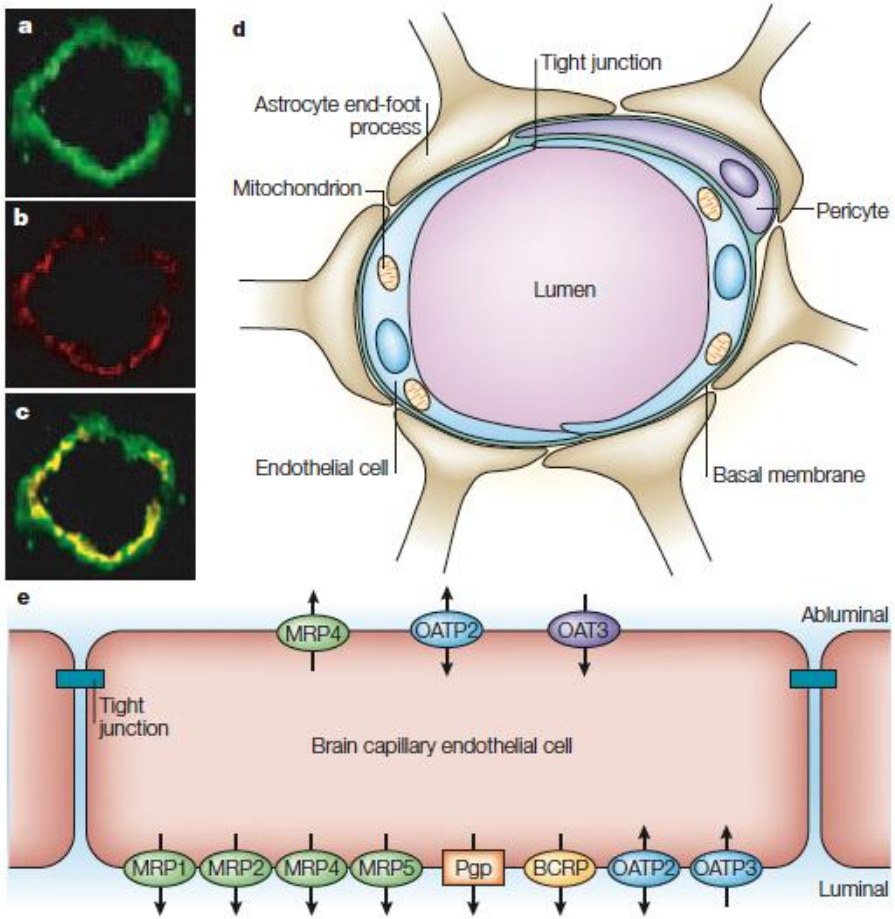
Perivascular glia, inflammatory mediators & brain microvasculature: New targets for intervention?

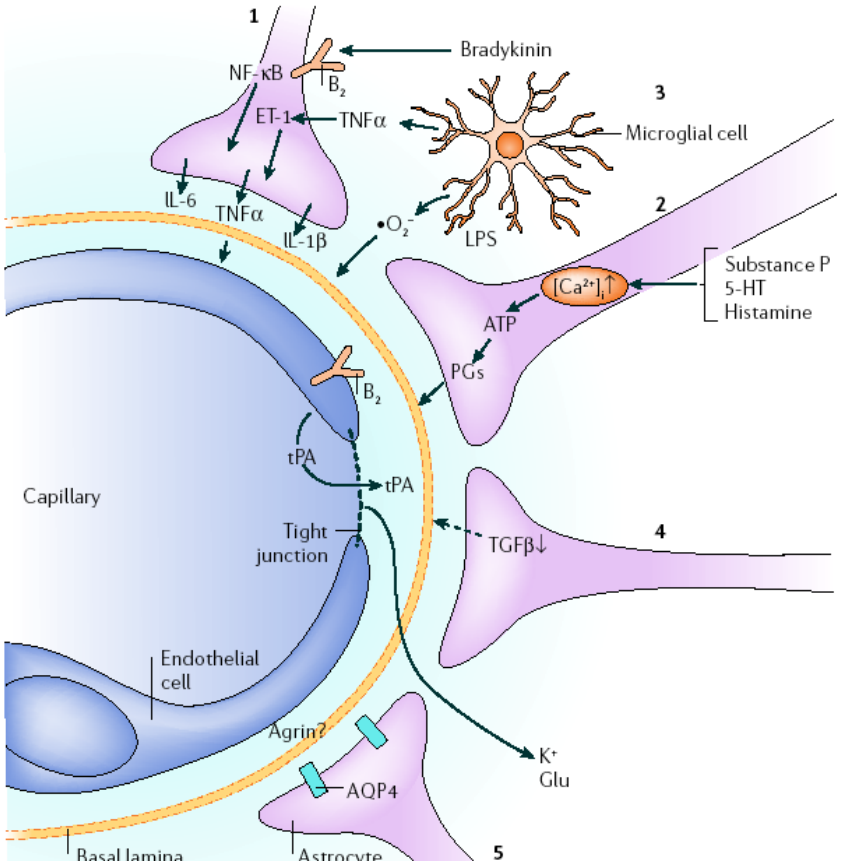
✓Neovascularization in CNS

✓Increase in BBB permeability

✓Induce adhesion molecules

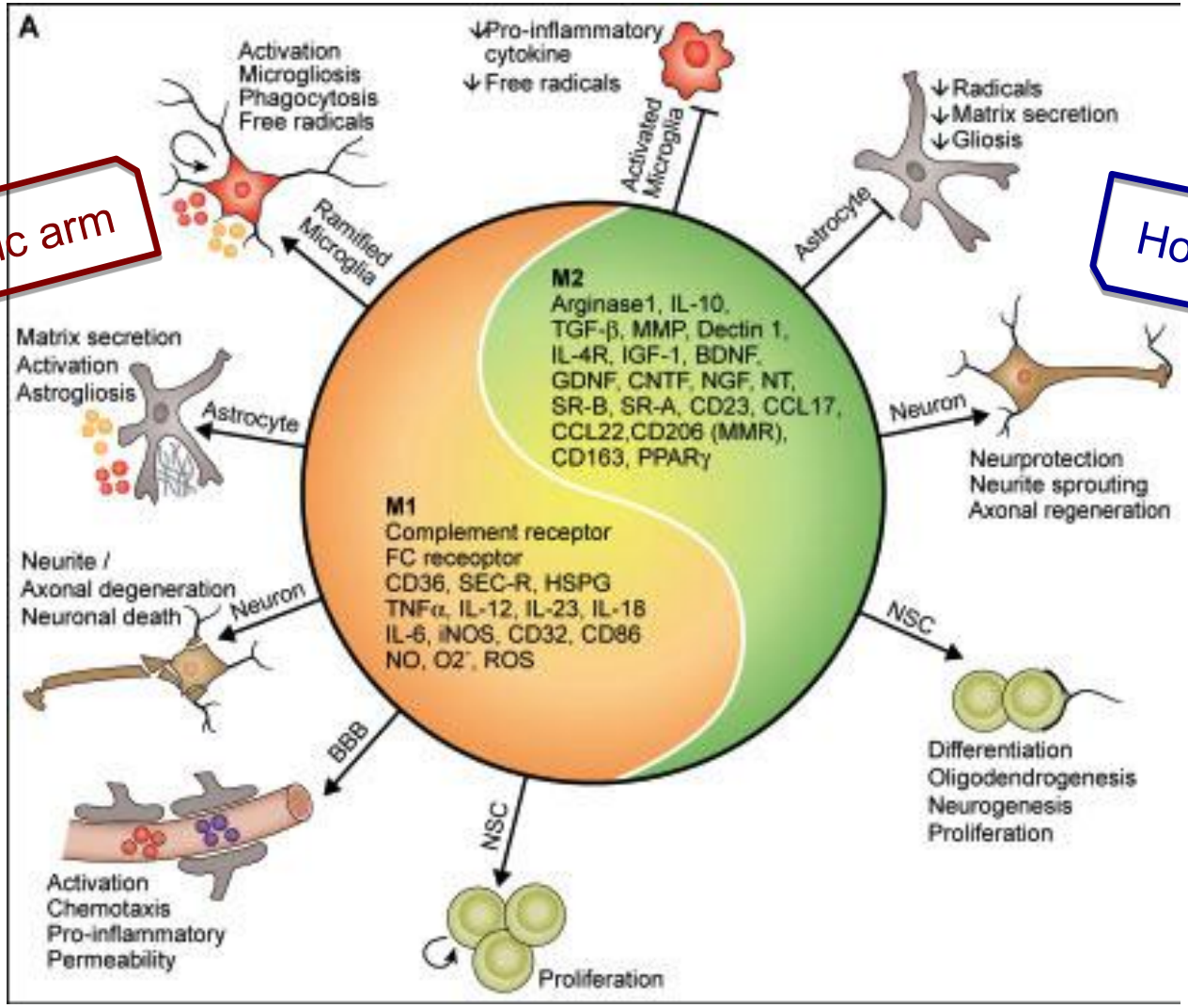
✓Induce MTP involved in pharmacoresistance (e.g. P-gp)





Loscher and Potschka, Nature Rev Neurosci, 2005

Harnessing microglia to control CNS inflammation?



Adapted from Shechter & Schwartz, J Pathol, 2013

Anti-inflammatory drugs as disease-modifying drugs

mTLE
MCD
RE

Symptomatic
Genetic (scarse info)

Precipitating insult

Trauma, Stroke, Seizures
Systemic/CNS Infection,
Autoimmunity, etc

Glia, Neurons,
Endothelial cells, Leukocytes

Inflammation

Cytokines, danger signals,
chemokines, complement,
Cox-2, CAM

DRUG

Astrocyte dysfunctions
Channelopathies

Neurotransmitters
HPA axis dysfunction
Vagal Nerve tone
BBB damage

Seizures
Cell loss
Gliosis
Molecular plasticity

Epilepsy

Neuropsychiatric
comorbidities

Depression
Autism
Memory deficits

Vezzani et al, Nature Rev Neurol, 2011; Exp Neurol, 2011